

## Autoimmune disorders and lymphoma

F. Caligaris-Cappio<sup>1</sup>

<sup>1</sup>Oncology, Università Vita-Salute San Raffaele, Istituto Scientifico San Raffaele, Milano, Italy

Considering that the cells involved in both autoimmunity and lymphoid malignancies are essentially the same, that a number of animal models have provided intersecting information, that the treatment options are frequently overlapping and that the immunotherapy of lymphoid tumours is based upon the manipulation of the immune system to obtain an anti-lymphoid tumour response, it is not surprising that the relationships between autoimmunity and lymphoid malignancies have long fascinated and puzzled both biologists and clinicians [1, 2]. Still our understanding of these relationships is marred not only by the very large gaps of knowledge in both fields, but also by the different and variable cultural approaches that frequently prevent a proper distinction of what is association from what is anecdotal evidence. Autoimmunity experts and lymphoma experts have different angles of view. One reason is the heterogeneity of the manifestations broadly labelled as 'autoimmune' which carries the inherent risk of lacking stringently defined inclusion criteria. This is exemplified by the use of serologic autoimmune parameters without a clear clinical correlate to define the presence of autoimmunity rather than the definition of autoimmune diseases on the basis of well-defined and internationally standardized criteria such as the ARA criteria. In biological terms the most critical and unsolved question remains the relationship between the events of cell activation promoted by auto-antigen (auto-Ag) stimulation and the events that lead to the malignant transformation of lymphoid cells.

All these difficulties taken into account, three major questions may be raised. (i) Is autoimmunity a predisposing factor to the development of lymphoid malignancies? (ii) Are patients affected by lymphoid tumours presenting with increased frequency autoimmune diseases and conversely are those with autoimmune diseases more frequently developing lymphoid malignancies? (iii) Which are the common themes in pathogenetic terms, i.e. which is the driver, which is (are) the passenger(s)?

### autoimmunity and lymphoid tumors: the lesson of epidemiology

A number of retrospective epidemiological studies have assessed that the development of lymphoid malignancies is

more common in patients with autoimmune diseases than in the general population. A personal or family history of a number of autoimmune diseases is strongly associated with increased risk of non-Hodgkin (NH) and Hodgkin's (H) lymphoma (L) and appears also to favour the development of monoclonal gammopathy of unknown significance (MGUS) and of overt multiple myeloma (MM) [3–5]. The range of autoimmune disorders involved is broad and variable. Overall it can be concluded that in first place primary Sjogren's syndrome (SS), but also rheumatoid arthritis (RA) and to a lesser extent systemic lupus erythematosus (SLE) have a high risk of NHL development [6]. The association between systemic autoimmune disorders treated with immunosuppressive drugs and an increased incidence of NHL is especially evident in RA where at least in some instances a role for Epstein–Barr virus appears to be present. It is of interest that in RA the risk of developing NHL has been shown to be higher in patients treated with cytotoxic or biologic (e.g. anti-tumour necrosis factor) agents. It is possible that this observation reflects the behaviour of specific patients whose inflammatory process is particularly severe and long lasting [2, 3, 6, 7].

This observation reinforces the growing evidence that chronic antigen (Ag) stimulation is involved in the onset of some chronic B-cell malignancies especially of marginal zone origin [8]. Both microbial and self-Ags are implicated, the classical example being the development of gastric MALT lymphomas in the context of chronic *Helicobacter pylori* infection. Along the same lines it is more than reasonable to postulate that the persistent stimulation of the immune system by auto-Ag may underlie the development of lymphomas within salivary glands in patients with SS or within thyroid tissue in Hashimoto's thyroiditis [1]. In SS chronic B-cell stimulation by activated T cells that secrete a variety of cytokines leads to B-cell proliferation and ultimately to malignant transformation. The large majority of resulting lymphoid tumours are lymphomas affecting the MALT system and these are especially extranodal lymphomas that may transform into diffuse large B-cell lymphomas (DLBLs) [9]. All these observations underline the links and interactions that take place between infection/inflammation, autoimmunity and the development of lymphoid malignancies [1, 2]. It has to be defined which are the relationships between classic Ag stimulation (inflammation/infection), development of autoreactivity and lymphoid tumours (lymphomagenesis) with dysregulation of B-cell response and which is the no-return point considering that there are numerous examples of

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lymphoma associated with other infections. One prototype example is hepatitis C virus (HCV) infection frequently but not necessarily accompanied by essential mixed cryoglobulinaemia where both large cells and indolent (usually marginal zone extranodal lymphomas) may develop [10] with a preferential localization in the liver and salivary glands (both target organs of HCV infection).

Chronic lymphoid malignancies of B-cell type have a striking association with organ-specific autoimmune diseases especially autoimmune haemolytic anaemia (AHA), idiopathic thrombocytopenia (ITP) and the rarer autoimmune neutropenia. AHA and ITP frequently complicate lymphoid malignancies such as chronic lymphocytic leukaemia of B-cell type (CLL) and are part of the clinical features of the autoimmune lymphoproliferative syndrome (ALPS) [1]. In this context CLL adds another intriguing element to the puzzle as it entails a uniquely profound and still unexplained perturbation of residual normal immune cells that leads to the progressive development of hypogammaglobulinaemia and to the occurrence of pathogenic auto-antibodies (Abs), which are polyclonal, hence produced by normal residual B cells. Surprisingly CLL pathogenic auto-Abs target virtually only Ag expressed by hematopoietic-derived cells, especially red cells and platelets [11], while systemic autoimmune disorders are exceptional. Abnormalities of specific T-cell subsets may explain the progressive development of hypogammaglobulinaemia and the generation of pathogenic polyclonal auto-Abs [12]. The reason why these auto-Abs are only causing autoimmune blood disorders remains a mystery.

MM paraproteins may be directed against a wide variety of infectious agents, suggesting that the development of MM may be causally related to chronic Ag stimulation. The observation that MM is a neoplasm of plasmablasts—plasma cells that have a post-switch phenotype (IgG or IgA), show somatic mutations of IgVH genes and may produce monoclonal Ig with targeted Ab activity suggests that the evolution of MM is an Ag-triggered process where germinal centres (GCs) of peripheral lymphoid organ(s) have a central role, even if the specific causal Ag is generally unknown. An interesting difference has been observed between MM and MGUS, as ongoing somatic mutations are present in MGUS, but not in MM, suggesting that MM is an Ag-selected, while MGUS is an Ag-driven process [1].

In some instances paraproteins of MM and of Waldenstrom's macroglobulinaemia (WM) have auto-Ab activity and may react with self-Ag usually represented by nerve constituents, Ig determinants, red blood cell antigens and clotting factors. The clinical consequences of this autoreactivity are numerous and include peripheral neuropathy most frequently due to reactivity against myelin-associated glycoprotein (MAG), Ab activity against the Fc receptor of Ig with formation of rheumatoid factor (RF, known as type II cryoglobulinaemia) that causes an immune-complex-mediated small vessel vasculitis, reactivity with group I/i antigens on the surface of red cells (cold agglutinin activity) that causes a chronic haemolytic anaemia and reactivity against clotting factors that causes coagulation abnormalities.

From the discussion so far developed it becomes evident that the available information concerns essentially the relationships

between autoimmune phenomena and B-cell lymphoid malignancies. Still the rare large granular lymphocyte (LGL) expansions, that belong mainly to the cytotoxic T-lymphocyte (CTL) subset, are observed in various clinical conditions, where they possibly represent a reaction of the immune system against an underlying Ag stimulation, and are often associated with autoimmune manifestations.

## B-cell development and growth: where it all starts

Most mature B lymphocytes are small, inactive cells whose specialized function is triggered by the encounter of their Ag receptor (B-cell receptor, BCR) with the complementary Ag. Mature B lymphocytes express a unique BCR on the cell surface that, after recognition of a specific antigen, triggers a cascade of signalling events which lead to cell activation, survival and differentiation by activating a cascade of protein kinases that eventually direct distinct transcription factors to enter the nucleus and to regulate gene expression [8]; the BCR also mediates antigen processing and presentation. In immature B lymphocytes BCR signalling can also trigger an apoptotic programme that eliminates potentially autoreactive B lymphocytes. The fate of the cells depends on the activity of coreceptors and on the affinity and amount of Ag. Actually a broader definition of the BCR signalling complex also includes coreceptors and immune inhibitory receptors that modulate its activity in a positive or negative manner. The pairing of activation and inhibition is necessary to modulate the immune response. Membrane-associated CD19, CD21 and CD81 take part in the BCR proximal signalling cascade and lower the threshold of Ag required for BCR to signal. The main inhibitory receptors in normal and leukaemic cells are CD5, CD72, CD22 and FC $\gamma$ RIIb. Three concurring signals are required for human B-cell maturation, namely BCR ligation, T-cell help and toll-like receptor (TLR) costimulation. TLR [13] can be engaged concomitantly with the BCR, at least in the context of autoimmune reactions where the dual engagement appears to be crucial for B-cell activation. In addition, TLR recognizes a set of different microbial components with some degree of specificity and acts as a co-stimulatory signal triggering an immediate innate immune response that induces B-cell maturation, proliferation and antibody production after pathogen recognition. The BCR is the key to B cell behaviour, and is unusual in having no unique ligand [8]. The level of BCR engagement therefore varies, being modulated by antigenic valency, epitope density and epitope organization. The threshold required for effective signalling, and the downstream outcome, also differ. Memory B cells also respond more rapidly to T-cell help. The initial strength of engagement may determine whether response is T-cell independent or requires T-cell help. Provision of T-cell help to a mature B cell is critical for generating high affinity antibody. If present, proliferation and differentiation can proceed. If absent, options include apoptosis or anergy.

The B-cell genome is reshaped throughout the B-cell life, especially in the proliferative phases that occur within the bone marrow (BM) and in the GC of secondary lymphoid follicles

when the intrinsic risk of mutational events is high. Likewise, rearrangements and mutations of Ig genes occur in the BM and in the GC where two major genetic events take place, the somatic hypermutation of Ig genes that allows the insertion of point mutations into Ig genes to produce Abs of increased affinity and the isotype switch that enables the production of Ig with the same Ag-binding specificity, but different functions. As receptor specificity is crucial, it is likewise essential to prevent the development of B cells expressing 'autoreactive' receptors [1, 2]. This censure is exerted either by removing the autoreactive cell by apoptosis or by 'editing' the autoreactive receptor, i.e. replacing one L chain by another L chain which is 'innocent'.

As the differentiation of lymphoid precursors is Ag independent, direct antigenic stimulation is not involved in the genesis of acute lymphoblastic leukaemia. On the contrary, as the very *raison d'être* of mature B lymphocytes is based upon their Ag receptor, the development of malignancies of mature B cells is likely favoured by antigenic stimulation. Heritable factors like variations in HLA and other genes encoding proteins involved in the immune response may behave as predisposition loci and account for an increased risk for lymphoid malignancies as well as for the development of autoimmune disease.

Normal B cells develop, exert their function or simply survive in specific microenvironments, compilation of cells that through cell-cell contacts and active molecular crosstalk provide individual organs with complex functional scaffoldings. All physiological events that involve B cells, from Ag encounter/presentation to B-cell activation, proliferation and differentiation, occur in specialized anatomical microenvironments where B cells are brought into intimate contact with T and accessory cells. Classically, human T-cell-dependent responses take place in GC of secondary lymphoid follicles in the presence of follicular dendritic cells (FDCs) and Ag-specific CD4<sup>+</sup> T cells. It is essentially in this site that somatic mutation of IgV genes, antigen selection and isotype switch events occur.

In blood cancers the relationships between transformed cells and microenvironment frequently play a central role [14]. Many indolent lymphomas retain the original cellular architecture and the specialized association with other cell types displayed by their normal counterparts. This is especially true in follicular lymphoma, which retains and faithfully recapitulates the general structure of normal reactive follicles, including the presence of T cells and FDCs, even when it localizes in the BM and in non-lymphoid organs. It is also true in B-cell lymphomas of MALT tissue whose malignant cells maintain an association with epithelial cells.

## may CLL provide a clue to the common pathogenetic themes?

CLL is at the crossroads between autoimmunity and lymphoid malignancies [8] for a number of reasons. First it is becoming more and more evident that CLL cells might be selected by some sort of antigenic pressure. This possibility is corroborated by the observation that CLL cases have a biased use of certain

IGHV genes and that subsets of CLL cases carry closely homologous if not identical (so-called 'stereotyped') complementarity-determining region 3 (CDR3) sequences on heavy and light chains [15]. CDR3 is unique for each B lymphocyte and its progeny as it is the most variable portion of the Ig sequence that defines the specificity of any given BCR. The probability that two individual B cells express identical BCRs is extremely low ( $10^{-12}$ ), hence the remarkable BCR similarity detected in >20% of unrelated and geographically distant CLL cases [16] is virtually impossible to be accounted for by pure chance. Rather it indicates that the recognition of a limited set of Ags likely plays a central role in selecting the leukaemic clones and leads us to consider antigenic stimulation as an important promoting factor. Next it has become clear that in numerous stereotyped cases HCDR3 indicates homology with various autoreactive clones including anti-DNA, anti-rheumatoid factor or anti-cardiolipin antibodies [16]. It has also been shown that several recombinant antibodies cloned from CLL patients with stereotyped receptors are auto- or poly-reactive. These data indicate the importance of self-Ags in the natural history of at least some CLL cases and suggest that CLL may be triggered or facilitated in its evolution by an auto-Ag. Whether this implies that CLL may be considered some sort of 'monoclonal neoplastic autoimmune disease' and how this concept may be inscribed in the above-mentioned perturbation of the immune system still remains to be clarified. Finally, in the tissues affected by CLL the proliferation compartment is essentially represented by pro-lymphocytes and paraimmunoblasts that cluster to form the pseudofollicular proliferation centres (PC). PC are focal aggregates of variable size scattered in lymph nodes and to a lesser extent in the BM [14] and are the hallmark of CLL as they are not detected in any other B-cell malignancy. Of interest they are also observed in inflamed tissues of patients with systemic autoimmune/inflammatory disorders such as RA and multiple sclerosis, reinforcing the concept of auto-Ag stimulation in CLL and further supporting the relationship between CLL and autoimmune disorders.

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